(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif;

wherein the saponin adjuvant is a chemically modified saponin adjuvant.

76. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 75.

77. (new) The method of claim 19, wherein the saponin adjuvant is a chemically modified saponin adjuvant.

78. (new) The immune adjuvant composition as claimed in claim 27, wherein the CpG motif comprises TCCATGACGTTCCTGACGTT.

79. (new) The method as claimed in claim 57, wherein the CpG motif comprises TCCATGACGTTCCTGACGTT.

REMARKS

Claims 19, 24, 27, 28, 32, 54, 57 and 58 have been amended, and new claims 63-79 have been added. Upon entry of the present amendments, claims 19-32 and 49-79 will be pending and under active consideration. A copy of all the claims, as amended, is attached hereto as Exhibit B. The amendments are fully supported by the present specification, and do not represent new subject matter. Support for the amendment to claim 19 that excludes compositions wherein the only immunostimulatory oligonucleotide is part of the nucleic acid sequence of a DNA vaccine vector may be found on page 8, lines 17-18.

Applicants respectfully request entry of the amendments and remarks made herein into the file history of the present application.

A. **Rejections Under 35 U.S.C. § 102**

1. **The Rejection Over Urban et al.**

The Examiner rejected claims 19-21, 24, 27, 29-32, 49-51, 54, 57 and 59-62 as being anticipated by United States Patent No. 6,013,258 (Urban et al.). The Examiner states that Urban et al. teaches administration of the combination of a plasmid encoding an antigen with saponin or Quil A. The plasmid taught in Urban, according to the Examiner, inherently contains at least one unmethylated CpG dinucleotide, and at least one motif of 5'X1CGX23'. Thus, the Examiner contends that Urban et al. inherently teaches a composition comprising the combination of an immunostimulatory oligonucleotide and a saponin adjuvant, as well as the administration thereof.

All claims rejected by the Examiner over Urban et al. depend upon, either directly or indirectly, claim 19. Claim 19 has been amended to exclude compositions comprising a DNA vaccine and a saponin adjuvant in the absence of an immunostimulatory CpG oligonucleotide that does not comprise a DNA vaccine. Thus, amended claim 19 reads upon, for example, the combination of a saponin, a DNA vaccine, and an immunostimulatory CpG motif oligonucleotide, but would not read upon the combination of saponin or Quil A adjuvant and DNA vaccine comprising an immunostimulatory CpG motif oligonucleotide with no other immunostimulatory CpG motif oligonucleotide present. Neither the compositions as presently claimed, nor the use thereof, are disclosed by Urban et al. Accordingly, Applicants request that this rejection be withdrawn.

In addition, the newly added claims do not read upon any composition or use thereof disclosed in Urban et al. Newly added claims 63 and 64 require that the saponin adjuvant be QS-7, QS-17 or QS-18. These adjuvants are not disclosed in Urban et al.

Claims 65-68 require that the immunostimulatory CpG motif oligonucleotide be a modified oligonucleotide. Modified immunostimulatory CpG motif oligonucleotides are not disclosed in Urban et al.

Claims 69-72, 78 and 79 require that the immunostimulatory CpG motif oligonucleotide comprise either TCTCCCAGCGTGCGCCAT or TCCATGACGTTCCTGACGTT. Urban et al. does not disclose either of these oligonucleotide sequences.

Claims 73 and 74 require that the immunostimulatory CpG motif oligonucleotide be from 5-40 base pairs in length. The oligonucleotides disclosed in Urban et al, being plasmids and/or capable of coding for the expression of a protein antigen, are much longer than 5-40 base pairs in length.

Claims 75, 76 and 77 require that the saponin adjuvant be a chemically modified saponin adjuvant. Chemically modified saponin adjuvants are not disclosed by Urban et al.

Accordingly, the newly added claims are not subject to a rejection under 35 U.S.C. § 102 over Urban et al.

2. **The Rejection Over Sasaki et al.**

The Examiner rejected claims 19-24, 27, 29-32, 49-54, 57 and 59-62 as being anticipated by United States Patent No. 5,808,024 (Sasaki et al.). The Examiner states that Sasaki et al. teaches administration of the combination of a plasmid encoding an antigen with QS-21. The plasmid taught in Sasake, according to the Examiner, inherently contains at least one unmethylated CpG dinucleotide, and at least one motif of 5'X1CGX23'. Thus, Sasake et al. inherently teaches a composition comprising the combination of an immunostimulatory oligonucleotide and the saponin adjuvant QS-21, as well as the administration thereof.

All claims rejected by the Examiner over Sasake et al. depend upon, either directly or indirectly, claim 19. Claim 19 has been amended to exclude compositions comprising a DNA vaccine and a saponin adjuvant in the absence of an immunostimulatory CpG oligonucleotide that does not comprise a DNA vaccine. Thus, amended claim 19 reads upon, for example, the combination of a saponin, a DNA vaccine, and an immunostimulatory CpG motif oligonucleotide, but would not read upon the combination of QS-21 adjuvant and DNA vaccine comprising an immunostimulatory CpG motif oligonucleotide in the absence of an immunostimulatory CpG motif oligonucleotide not comprising a DNA vaccine. Neither the compositions as presently claimed, nor the use thereof, are disclosed by Sasake et al. Accordingly, Applicants request that this rejection be withdrawn.

In addition, the newly added claims do not read upon any composition or use thereof disclosed in Sasake et al. Newly added claims 63 and 64 require that the saponin adjuvant be QS-7, QS-17 or QS-18. These adjuvants are not disclosed in Sasake et al.

Claims 65-68 require that the immunostimulatory CpG motif oligonucleotide be a modified oligonucleotide. Modified immunostimulatory CpG motif oligonucleotides are not disclosed in Sasake et al.

Claims 69-72, 78 and 79 require that the immunostimulatory CpG motif oligonucleotide comprise either TCTCCCAGCGTGCGCCAT or TCCATGACGTTCCTGACGTT. Sasake et al. does not disclose either of these oligonucleotide sequences.

Claims 73 and 74 require that the immunostimulatory CpG motif oligonucleotide be from 5-40 base pairs in length. The oligonucleotides disclosed in Sasake et al, being plasmids and/or capable of coding for the expression of a protein antigen, are much longer than 5-40 base pairs in length.

Claims 75, 76 and 77 require that the saponin adjuvant be a chemically modified saponin adjuvant. Chemically modified saponin adjuvants are not disclosed by Sasake et al.

Accordingly, the newly added claims are not subject to a rejection under 35 U.S.C. § 102 over Sasake et al.

B. <u>Rejection 35 U.S.C. § 103</u>

Claims 19-32 and 49-62 were rejected under 35 U.S.C. § 103 as being unpatentable over Weiner et al., Sept. 1997, PNAS, Vol. 94, pages 10833-10837 ("Weiner") in view of Kensil, 1996, Critical Reviews in Therapeutic Drug Carrier Systems, Vol. 13, No. 1 and 2, pages 1-55 ("Kensil"). According to the Examiner, Weiner discloses an immunostimulatory CpG motif oligonucleotide with the sequence of SEQ ID NO: 1, but does not disclose the combination of such immunostimulatory CpG motif oligonucleotide and saponin. Kensil teaches the use of the saponin adjuvant QS-21 in combination with tumor antigens to enhance the immune response to said tumor antigen when administered to a subject. The Examiner contends that it would have been obvious to combine the two known adjuvants (the immunostimulatory CpG motif oligonucleotide with SEQ ID NO: 1 and QS-21), particularly in light of a teaching in Weiner that provides an invitation to experiment with combinations of immunostimulatory CpG motif oligonucleotides with other adjuvants.

Assuming, *arguendo*, that the cited references did make a *prima facie* case of obviousness, Applicant has demonstrated the unexpected result of synergism of immunostimulatory CpG motif oligonucleotides and saponin adjuvants, thereby rebutting any *prima facie* case of obviousness.

An Applicant may rebut evidence of obviousness, *i.e.,* a case of *prima facie* obviousness, of an invention by showing that the invention displays "unexpected results."

In re Dillon, 919 F.2d 688 (Fed. Cir. 1990) (*in banc*); *In re Malagari*, 499 F.2d 1297 (CCPA 1974), *In re De Blauwe*, 736 F.2d 699 (Fed. Cir. 1984); *In re Burckel*, 592 F.2d 1175 (CCPA 1979)). In this instance, the specification demonstrates that the combination of immunostimulatory CpG motif oligonucleotides and saponin has an effect on the immune response that is greater than the expected additive effect of immunostimulatory CpG motif oligonucleotides and saponin. Applicants direct the Examiner's attention to Figure 1 of the specification. Figure 1 depicts the effects of several different adjuvant compositions on antigen specific cytotoxic T cell mediated lysis of cells. As can be seen, 2, 10, and 50 μg of an immunostimulatory CpG motif oligonucleotide and 1.25 μg of QS-21 (administered alone, not in combination) were unable to stimulate significant cytotoxic T cell lysis. 10 μg of QS-21 was able to generate an intermediate effect. Surprisingly, the combination of, for example, 1.25 μg of QS-21 and 50 μg of immunostimulatory CpG motif oligonucleotide was able to generate significant cytotoxic T cell lysis. Alone, each component was unable to generate greater than approximately 5% lysis, but together, they were able to generate up to nearly 90% lysis. In fact, every combination of immunostimulatory CpG motif oligonucleotide and saponin adjuvant in Figure 1 had a synergistic effect greater than the expected merely additive effect. Additional evidence of synergism is depicted in Figures 2-9. It is unexpected that these two compounds should have any more than an additive effect. Thus, the evidence of synergism rebuts any possible *prima facie* case of obviousness, and the rejection under 35 U.S.C. § 103 should be withdrawn.

C. <u>Rejections Under 35 U.S.C. § 112</u>

Claim 32 was rejected because the term "antigen" has insufficient antecedent basis. Claim 32 has been amended, obviating the rejection.

Claims 29-31 and 59-61 were rejected for indefiniteness, because, according to the Examiner, it was unclear if Applicants were attempting to further limit the claim to administering a composition comprising the immunostimulatory CpG motif oligonucleotide and saponin and an antigen or to administering a composition comprising the immunostimulatory CpG motif oligonucleotide and saponin and increasing the immune response to an antigen that is administered to the mammal at a later time. Applicants intend the claim to cover either situation, as is described, for example, on page 18, lines 9-11 of the specification, where the composition of the invention is described as being administered "as a single injection of a mixed formulation of saponin, oligonucleotide and antigen, or as

separate injections given at the same site within a short period of time (*i.e.*, 0-2 days)." The Examiner's inability to discern which situation was meant to be covered indicates that the claim, as written, is definite, since both situations are meant to be within the scope of the claims. Thus, Applicants submit that claims 29-31 and 59-61 are definite, and do not require amendment for reasons of indefiniteness.

Claims 32 and 62 were rejected because, according to the Examiner, "antigens" comprise amino acid/polypeptide sequences and do not comprise proteins, peptides, polysaccharides, lipids, glycolipids, phospholipids or nucleic acid sequences. The Examiner contends that the terms used do not further limit the antigen. Applicants submit that, in fact, each of the recited molecules may function as an antigen, and therefore, recitation of each type of molecule is appropriate. The immune system is not limited to the recognition of only amino acid/polypeptide sequences. For example, an entire class of antigens, CD-1 antigens, are notable for not being peptide antigens. Thus, the recitation in claims 32 and 62 of different types of antigens is proper, and the rejection of claims 32 and 62 should be withdrawn.

Claims 27 and 57 were rejected as being indefinite because the phrase "wherein at least one nucleotide separates consecutive CpGs" is, according to the Examiner, unclear. This phrase has been removed from the claims by this amendment. Thus, the indefiniteness rejection of claims 27 and 57 should be withdrawn.

CONCLUSION

Applicants respectfully request that the amendments and remarks above be entered and made of record in the file history of the instant application. Applicants believe that each ground for rejection or objection has been successfully overcome or obviated and that the application is in condition for allowance. Early notification to this effect is earnestly solicited.

Respectfully submitted,

Date May 18, 2001

47,167

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Enclosure

19. An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide is not a part of the nucleic acid sequence of a DNA vaccine vector.

24. The immune adjuvant composition as claimed in claim 19, wherein the immunostimulatory oligonucleotide comprises [at least one] more than one unmethylated CpG dinucleotide.

27. The immune adjuvant compositing as claimed in claim 19 [24], wherein the immunostimulatory oligonucleotide comprises a CpG motif having the formula 5'X1CGX23', [wherein at least one nucleotide separates consecutive CpGs, and] wherein X1 is adenine, guanine, or thymine, and X2 is cytosine, thymine, or adenine.

28. The immune adjuvant composition as claimed in claim 27, wherein the CpG motif comprises TCTCCCAGCGTGCGCCAT[or TCCATGACGTTCCTGACGTT].

32. The immune adjuvant composition as claimed in claim 19 [27], [wherein the antigen comprises] further comprising an antigen selected from the group consisting of a protein, a peptide, a polysaccharide, a lipid, a glycolipid, a phospholipid, [or] and a nucleic acid encoding the protein or peptide.

54. The method as claimed in claim 49, wherein the immunostimulatory oligonucleotide comprises [at least one] more than one unmethylated CpG dinucleotide.

57. The method as claimed in claim 49 [54], wherein the immunostimulatory oligonucleotide comprises a CpG motif having the formula $5'X_1CGX_2 3'$, [wherein at least one nucleotide separates consecutive CpGs, and] wherein X_1 is adenine, guanine, or thymine, and X_2 is cytosine, thymine, or adenine.

58. The method as claimed in claim 57, wherein the CpG motif comprises TCTCCCAGCGTGCGCCAT[or TCCATGACGTTCCTGACGTT].

EXHIBIT B
THE CLAIMS WHICH WILL BE PENDING
UPON ENTRY OF THE PRESENT AMENDMENT
(Filed May 18, 2001)
U.S. PATENT APPLICATION SERIAL NO. 09/396,941

19. An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide is not a part of the nucleic acid sequence of a DNA vaccine vector.

20. The immune adjuvant composition as claimed in claim 19, wherein the saponin adjuvant is derived from *Quillaja saponaria*.

21. The immune adjuvant composition as claimed in claim 20, wherein the saponin adjuvant comprises a substantially pure saponin adjuvant.

22. The immune adjuvant composition as claimed in claim 21, wherein the substantially pure saponin adjuvant comprises QS-7, QS-17, QS-18, or QS-21.

23. The immune adjuvant composition as claimed in claim 22, wherein the substantially pure saponin adjuvant comprises QS-21.

24. The immune adjuvant composition as claimed in claim 19, wherein the immunostimulatory oligonucleotide comprises more than one unmethylated CpG dinucleotide.

25. The immune adjuvant composition as claimed in claim 19, wherein the immunostimulatory oligonucleotide is modified.

26. The immune adjuvant composition as claimed in claim 25, wherein the immunostimulatory oligonucleotide is modified with at least one phosphorothioate-modified nucleotide.

27. The immune adjuvant compositing as claimed in claim 19, wherein the immunostimulatory oligonucleotide comprises a CpG motif having the formula 5'X1CGX23', wherein X1 is adenine, guanine, or thymine, and X2 is cytosine, thymine, or adenine.

28. The immune adjuvant composition as claimed in claim 27, wherein the CpG motif comprises TCTCCCAGCGTGCGCCAT.

29. The immune adjuvant composition as claimed in claim 19, wherein the composition increases the immune response to an antigen when administered to a mammal.

30. The immune adjuvant composition as claimed in claim 19, wherein the composition increases the immune response to an antigen when administered to a human.

31. The immune adjuvant composition as claimed in claim 19, wherein the composition increases the immune response to an antigen when administered to a animal.

32. The immune adjuvant composition as claimed in claim 19, further comprising an antigen selected from the group consisting of a protein, a peptide, a polysaccharide, a lipid, a glycolipid, a phospholipid, and a nucleic acid encoding the protein or peptide.

49. A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 19.

50. The method as claimed in claim 49, wherein the saponin adjuvant is derived from *Quillaja saponaria.*

51. The method as claimed in claim 50, wherein the saponin adjuvant comprises a substantially pure saponin adjuvant.

52. The method as claimed in claim 51, wherein the substantially pure saponin adjuvant comprises QS-7, QS-17, QS-18, or QS-21.

53. The method as claimed in claim 52, wherein the substantially pure saponin adjuvant comprises QS-21.

54. The method as claimed in claim 49, wherein the immunostimulatory oligonucleotide comprises more than one unmethylated CpG dinucleotide.

55. The method as claimed in claim 49, wherein the immunostimulatory oligonucleotide is modified.

56. The method as claimed in claim 55, wherein the immunostimulatory oligonucleotide is modified with at least one phosphorothioate-modified nucleotide.

57. The method as claimed in claim 49, wherein the immunostimulatory oligonucleotide comprises a CpG motif having the formula 5'X_1CG$X_2$3', wherein X_1 is adenine, guanine, or thymine, and X_2 is cytosine, thymine, or adenine.

58. The method as claimed in claim 57, wherein the CpG motif comprises TCTCCCAGCGTGCGCCAT.

59. The method as claimed in claim 49, wherein the composition increases the immune response to an antigen when administered to a mammal.

60. The method as claimed in claim 49, wherein the composition increases the immune response to an antigen when administered to a human.

61. The method as claimed in claim 49, wherein the composition increases the immune response to an antigen when administered to an animal.

62. The method as claimed in claim 59, wherein the antigen comprises a protein, a peptide, a polysaccharide, a lipid, a glycolipid, a phospholipid, or a nucleic acid encoding the protein or peptide.

63. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the saponin adjuvant comprises substantially pure QS-7, QS-17 or QS-18.

64. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 63.

65. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide is modified.

66. (new) The immune adjuvant composition as claimed in claim 65, wherein the immunostimulatory oligonucleotide is modified with at least one phosphorothioate-modified nucleotide.

67. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 65.

68. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 66.

69. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide comprises TCTCCCAGCGTGCGCCAT.

70. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 69.

71. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide comprises TCCATGACGTTCCTGACGTT.

72. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 71.

73. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif,

wherein the immunostimulatory oligonucleotide is from 5-40 base pairs in length.

74. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 73.

75. (new) An immune adjuvant compositing comprising

(a) a saponin adjuvant; and

(b) an immunostimulatory oligonucleotide comprising at least one unmethylated CpG motif;

wherein the saponin adjuvant is a chemically modified saponin adjuvant.

76. (new) A method for increasing the immune response to an antigen in an individual or a test system to which the antigen is administered comprising administering an effective amount of an immune adjuvant composition as claimed in claim 75.

77. (new) The method of claim 19, wherein the saponin adjuvant is a chemically modified saponin adjuvant.

78. (new) The immune adjuvant composition as claimed in claim 27, wherein the CpG motif comprises TCCATGACGTTCCTGACGTT.

79. (new) The method as claimed in claim 57, wherein the CpG motif comprises TCCATGACGTTCCTGACGTT.